特別掲載

Effect of X-ray Radiation and Mitomycin C Administration on Cell Cycle of Yoshida Sarcoma

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吉田腹水肉腫の細胞周期に及ぼすX線照射マイトマイシンC投与の影響

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先に吉田腹水肉腫を Wister rat に移植後4日目の generation time (T) を autoradio-graphical に測定し約19時間である事を報告したが，今回はX線照射及びマイトマイシンC投与がTに及ぼす影響を autoradiograph 法にて観察した．

X線照射群は 200R，1000Rを1回腹部に照射し，マイトマイシンC投与群は10γ， 100γを腹腔内1回投与して吉田腹水肉腫のTの変動を観察した．X線照射群では 200R，1000Rともに G_2-delay が見られ，Tの延長は見られなかつた．マイトマイシンC10γ 1回投与では G_2-delay 及びTの延長が見られ， 100γ投与では G_2-delay 及び著しいTの延長があると思われた．これは細胞の破壊が強く72時間の測定では第2の peak が出現しなかつたために延長時間が測定出来なかつた．X線照射群，マイトマイシンC 投与群共に G_2-delay が見られ前者ではTの延長は見られず，後者ではTの延長が見られた．

During the last 10 years many reports have been published on the effect of ionizing radiation on the cell cycle of malignant tumor. In these reports cell division and DNA synthesis are used as the measures of the effect, and relation and comparison between these two are discussed[1,2,4,5,6,7,9,10,11,12,13,15,16,17,19] In our experiments Yoshida ascites sarcoma (hereafter referred to as Y.S.) received X-ray irradiation or mitomycin C (MMC) administration, and their effects on the cell cycle were investigated.

Experimental materials and methods

1. In vivo experiments

1) 200 R irradiation: Wister rats received intraperitoneal transplantation of 10^7 cells of Y.S., and 4 days later they were intraperitoneally injected with 100 μCi of ^{3}H-thymidine (3H-TDR) at a activity of 2.5 Ci/mM and one hour thereafter irradiated on the whole body with 200 R (Toshiba KXC-18, 150 KVP, 25 mA, filter Cu 0.5 mm+Al 0.5 mm, H.V.L. Cu 0.86 mm, F.S.D. 30 cm, 94 R/min). Every 2 hours, ascitic fluid was drawn to make smear samples, from which autoradiograms were prepared by the stripping method. The percentage of labeled metaphase of each sample was plotted against time. We used three or more animals in each experiment.

2) 1000 R irradiation: The same procedure was followed after irradiation with 1,000 R.

2. In vitro experiments

1) 200 R irradiation: On 4 days after transplantation of 10^7 cells of Y.S. rats were irradiated on the whole body with 200 R (under the same condition as in Experiment 1). Ascitic fluid was drawn by puncture every 2 hours, incubated with 0.02 μCi/5 × 10^4 cells of 3H-TDR (specific activity 2.5 Ci/mM) and 1 ml of plasma, in a water bath at 37.5 °C for 20 minutes to make labeling. After washing the cells (centrifuged at 1,000 r.p.m for 5 minutes), smear samples were prepared, from which autoradiograms were made by the stripping method. Both the labeling index (l.i.) and mitotic index (m.i.) of the samples were determined.

2) 1,000 R irradiation: After the whole body irradiation at 1,000 R under the same condition as in experiment 1, the same procedure was followed as in experiment 2. 1).

3. MMC Experiments

1) 10 γ administration: On 4 days after intraperitoneal transplantation of 10^7 cells of Y.S., the animals were intraperitoneally injected with 100 μCi of 3H-TDR (specific activity 2.5 Ci/mM), and one hour later with 10 γ of MMC. Then ascitic fluid was taken every two hours by puncture in the course of 1—72 hours, and from their smear samples autoradiograms were prepared. And labeled metaphase and m.i. were observed.

2) 100 γ administration: The same procedure as in experiment 3. 1), was followed after administration of 100 γ.

Results

1. In vivo experiments

1) 200 R irradiation (Fig. 1): A curve in the percentage of labeled metaphases was drown by free hand. Labeled metaphase began to appear at 3 hours after the injection of 3H-TDR, and then gradually increase, attaining the maximum 98% at 17 hours. Then gradual decline commenced to reach the minimum at 31 hours, and again increase to reach the second peak at 36 hours, followed by a fall once more. Compared with the control group[18], the appearance of labeled metaphase was delayed about 1.5 hours. The increasing rate of labeled metaphases was similar to that of the control in the beginning, but became gradually smaller, and the first peak was attained about 7 hours later than in the control. The same was true also with the second peak. As for the duration of each phase of the cell cycle obtained from this curve, G_2 lasted 7 hours, and S 21 hours, both being prolonged as compared with the controls. But the generation time, T, was 19 hours, not prolonged. The m.i. was

Fig. 1 Percent of labeled metaphase cells at various intervals after injection of ^{3}H-TDR

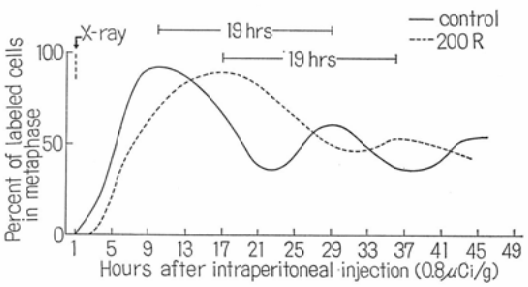
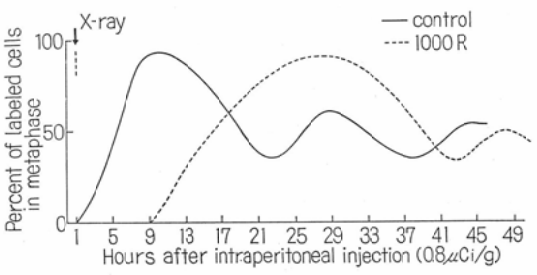


Fig. 2 Per cent of labeled metaphase cells at various intervals after injection of ^{3}H-TDR



decreased immediately after irradiation, and attained a minimum at 3 hours. But thereafter it increased returning to the initial level at about 6 hours.

2) 1,000 R irradiation (Fig. 2): Labeled metaphase began to appear at 9—10 hours after 3H-TDR injection, and gradually increased showing a rather gentler slope than after 200 R irradiation. The first peak (92%) was reached at about 29 hours, and thereafter the curve assumed nearly the same tendency as in 1), the first valley being seen at about 43 hours, and the second peak at about 49 hours. The appearances of these points were each delayed about 19 hours as compared with the control. G_2 and S phases were remarkably prolonged, being 15.5 and 23.5 hours, respectively. T was 19 hours, not prolonged. The m.i. began to decrease rather sharply soon after 1,000 R irradiation, became 0 at 2 hours, and after keeping this state, began to increase at 9 hours. After rise it returned to the initial level at about 18 hours.

2. In vitro experiments

1) 200 R irradiation (Fig. 3): Determination of l.i. was started one hour after the irradiation. It began to diminish immediately, attained the bottom at 6 hours, and after keeping this state for 6 hours, began to rise gradually, returning to the initial value at about 18 hours. The lowest l.i. was about that is 53% of the initial value. The m.i. was the same as in Experiment 1, 1).

Fig. 3 Invitro incorporation of ^{3}H-TDR in cells after whole body irradiation with 200R

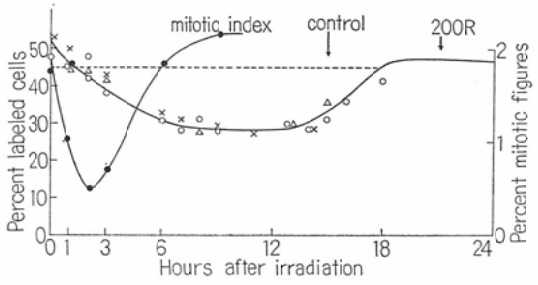
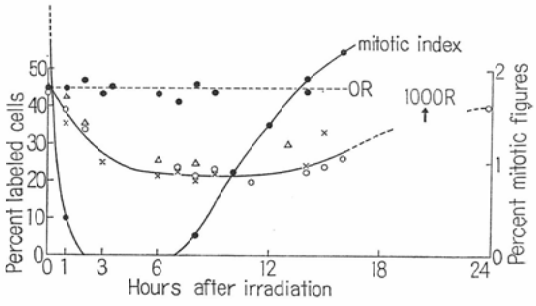


Fig. 4 Invitro incorporation of ^{3}H-TDR in cells after whole body irradiation with 1.000R



2) 1,000 R irradiation (Fig. 4):

After l.i. of the cells in this group decreased sharply below the minimum in the case of 200 R irradiation l.i. reached minimum. Then l.i. increased much the same as the process of the 200 R group.

3. MMC Experiments

1) 10 γ administration (Fig. 5): In the MMC 10 γ group, labeled metaphase began to appear at about 3 hours after 3H-TDR injection, continued a slow rise thereafter, attaining the first peak (90%) at about 24 hours. Then gradual decline followed, and after a valley was reached at about 52 hours, elevation again continued until the second peak was reached at about 61 hours. The generation time, T, G_1, S and G_2 +M phases were 37, 7, 28 and 2 hours, respectively. These are prolonged ad compared with T at 4 days after Y.S. transplantation and the durations of the phases which were given in the previous report; G_2 was prolonged 1—1.5 hours, and T 18 hours. The m.i. immediately fell after MMC administration, attained the minimum of about 10‰ at 8 hours, and then gradually returned nearly to the initial level at 48 hours.

Fig. 5 Per cent of labeled metaphase cells at various intervals after injection of ^{3}H-TDR

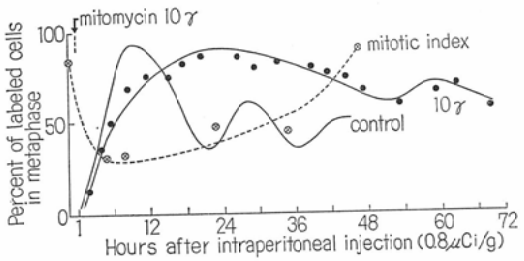
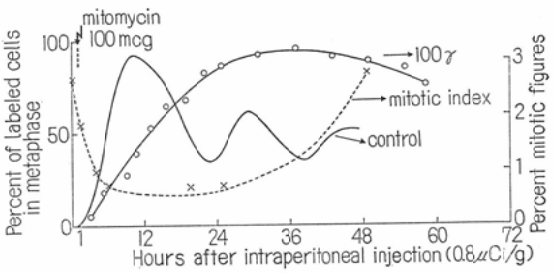


Fig. 6 Percent of labeled metaphase cells at various intervals after. injection of ^{3}H-TDR



2) 100 γ administration (Fig. 6): In this group, labeled metaphase began to appear at about 3 hours after 3H-TDR injection, and increasing more slowly than that of 10 γ group, attained the first peak (95%) at about 36 hours. Then gradual decrease followed. At about 60 hours, however, serious diminution of the tumor cells took place, so that observation became impossible G_2 prolonged 1—1.5 hours. The m.i. showed abrupt decrease after MMC administration, attained the minimum of 6‰ at 7 hours, and maintained this value about 20 hours thereafter. Then it increased gradually, returning the initial level at about 48 hours.

Discussion

What can immediately be seen in Figs. 1 and 2, is that the first peak in the labeled metaphase curve was shifted toward the right so much as the dose of the irradiation increased, and that the slope of each rise became gentler. This is to say that the durations of G_2 and S phases were prolonged by the irradiation (without irradiation, 5.5 and 12.5 hours; with 200 R, 7 and 21 hours; with 1,000 R, 15.5 and 23.5 hours, respectively). Since it is inconceivable that two kinds of metabolism should be performed in G_2 or S phases of one cell, the above mentioned facts seem to indicate that the two phases are particularly radiosensitive.

Painter et al[11,12,16] and Kim and Evans[7] observed, in He-La S_3 carcinoma cells and Ehrlich ascites tumor cells, respectively, that l.i. increased for several hours after 500 R irradiation as the result of inhibition of DNA synthesis. In our present experiments shown in Figs. 3 and 4. decrease of the l.i. started immediately after irradiation, and such increase was not found either with 200 R or 1,000 R irradiation. This is considered to result from shorter duration of G_1 phase of Y. S. than those of He-La S_3 carcinoma and Ehrlich ascites tumor. Our results showed that in both cases, increase of the l.i. began at about 12 hours following the irradiation after considerably low values lasting about 6 hours, and that the initial value was restored at about 18 hours. Kim and Evans[7] obtained durations of M and G_1 phases by the Stanner and Till's method[14], and measured duration of mitotic inhibition in irradiation experiments. From these values they estimated the starting times of decrease of l.i. following transient (several hours) increase after irradiation and of its succeeding increase. They found very good agreement between the estimated and the measured values. It was, however, impossible to apply their quantitative interpretation to our results. This discordance between theirs and ours can not be attributed merely to difference in animal tumor, because the duration of S phase of Ehrlich ascites tumor (8.5 hours by the Stanner and Till's method, and 8.8 hours by practical measurement) corresponds well with that for

Y.S. (9 hours by the Stanner and Till's method). But the measured value of the latter is 12.4 hours. This difference of 3.4 hours is considered to be the cause of the above mentioned discordance. It is worth to notice in this case that the measured and the computed value are in good agreement if we assume that the phases of Y.S. or AH-13 have log-normal distribution independent from one another as seen in our report on g.t. of Y.S.[18] Kozuka and Moor[8], who made cinematographic as well as autoradiographic studies on culture of He La cells, observed that the duration of each phase varied widely in all the cells, and that there was difference even between two daughter cells from the same mother cell. This fact gives important reason why certain reservation must be made in attempting to analyze experimental results on the hypothesis that all the cells constituting a population have the same phase duration.

The only justifiable view at this moment, though not yet proved by evidences, which can evade confusion resulting from varying experimental results, seems to be based on the hypothesis that the duration of each phase of Y.S. cell has independent log-normal distribution, and consequently that standard deviation are increased continuously even in non-irradiated cells, that is, in spontaneous process after transplantation(in other words, asynchronous progress becomes faster with the lapse of time), and that the progress is accelerated further by irradiation. This can easily be understood by finding, in our non-irradiation experiment, the second peak low and flat, and by seeing, in Fig. 5 in Kim and Evans paper[7], the difference between the curve for the non-irradiated control and that for irradiated group. Next, our results showed that the interval between the first and the second peak seemed to be unchanged whether the irradiation dose was 200 R or 1,000 R. It is contradictory that this interval should remain unaltered despite prolongation of G_2 and S phases. If we should explain this at all we would say that the acceleration of the asynchrony, brought about by irradiation, might be abolished by recovery from damage of irradiation, and that there might be no damaged cells among labeled mitotic cells since they would already have terminated cell division. In the same way can be explained the fact that the time necessary for l.i. to return to the initial level after decrease by irradition was about 18 hours in both 200 R and 1,000 R doses. But for the moment this can not be any more than more estimation. Figs. 5 and 6. represent the effect on cell cycle of MMC, which is a DNA synthesis inhibitor, and which is clinically used as a most potent anti-cancer agent. The curve is closely similar to that after irradiation, but indicates greater effect. However, G_2 after administration of 100 γ of MMC (7 hrs) was less prolonged than after 1,000 R irradiation, and nearly equal to that after 200 R irradiation. But despite this, the interval between the first and the second peak was far longer after 10 γ administration than after 200 R irradiation. This indicates that while the effect of irradiation is abolished in a short time, that of MMC persists long, and that the action mechanism of the latter is inhibition of DNA synthesis[3]. Consequently, the effect of MMC is slight on G_2 phase and profound on S phase. The results obtained with MMC as well as those obtained by irradiation can be explained by the view that in Y.S. asynchrony tendency is naturally accelerated, that the duration of each phase has its own independent probability distribution, that it responds to the applied damage by dispersion of its distribution, and that the change produced is reversible. Now we are performing experiments with the purpose to substantiate the above discussion.

Summary

Previously we determined generation time of Yoshida ascites sarcoma by autoradiography (with

^{3}H-TDR)[18]. In order to see whether there is any difference in action mechanism between X-ray and mitomycin C, which are now clinically employed we measured T of Y.S. and observed each phase of its growth. In X-ray irradiation we found G_2-delay and prolongation of S phase, but not prolongation of generation time. In mitomycin C injection, however, we observed prolongation of both T and S phase as well as G_2-delay. This means that while X-ray gives transient damage to Y.S., mitomycin C exerts persistent effect. Prolongation of generation time by the latter can be ascribed to inhibition of DNA synthesis.

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